

C L A I M S

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1. A process for the qualitative and quantitative determination of at least one in vitro amplified nucleic acid in a sealed reaction chamber,
 - wherein during or subsequent to the amplification of the nucleic acid at least one ^{probe} substance (probe) is present which (interacts) with the nucleic acid to be detected;
 - wherein spectrophotically measurable parameters of said substance (probe) are subject to variation, creating a measurable signal;
 - wherein the sample to be measured is exposed to the action of a gradient capable of at least partially denaturing nucleic acids;
 - with detection of the measurable parameter undergoing variation through the action of the gradient; and
 - the entire amplification reaction, including qualitative and quantitative determination, may be carried out in a sealed reaction chamber (measuring compartment) without intermittent opening.
 2. The process according to claim 1, wherein the probe, the spectrophotically measurable parameter of which is at least one luminescent or fluorescent dye, contains a nucleic acid proportion, the interaction of which with the in vitro amplified nucleic acid as a function of the denaturation condition is accompanied by a change in the spectrophotically measured signal, preferably, by intercalation of the dye into the nucleic acid

double helix or by dilution or concentration effects within the measuring compartment.

a 3. The process according to ~~one of claims 1 or 2,~~ ^{claim 1} wherein a denaturation process is detected using wave length variation and/or shift in luminescence or fluorescence intensity and/or variation in fluorescence polarization and/or variation in excited state lifetime, or using the principle of the so-called "energy transfer", or through a concentration effect.]

a 4. The process according to ~~at least one of claims 1 to 3,~~ ^{claim 1} (characterized in that) in a reaction chamber a multiplicity of dyes are employed which may be distinguished from each other spectroscopically and which permit to analyze the various amplified nucleic acids and/or through which at least one independent calibrating substance may be introduced.

a 5. The process according to ~~at least one of claims 1 to 4,~~ ^{claim 1} characterized in that luminescence, in particular, fluorescence of the dyes is excited continuously or in pulses by a laser.

a 6. The process according to ~~at least one of claims 1 to 5,~~ ^{claim 1} characterized in that the amplified nucleic acids contain at least one co-amplified nucleic acid standard, the sequence of which is homologous to a sequence to be determined, preferably identical, with the exception of at least one point mutation which, preferably, lies in a sequence region of lowest stability but outside the primer binding sites in enzymatic amplification.

a 7. The process according to ~~at least one of claims 1 to 5,~~ ^{claim 1} characterized in that the amplified nucleic acids contain at least one co-amplified nucleic acid standard, the sequence of which is absolutely homologous in the

primer regions, but otherwise the sequence of the nucleic acid standard deviates from the sequence of the amplified nucleic acid in more than one position.

a 8. The process according to at least ~~one of claims 1 to 7~~, characterized in that in part, the nucleic acid standard itself is a natural component of the nucleic acid to be analyzed.

a 9. The process according to at least ~~one of claims 1 to 8~~, characterized in that amplification is carried out in homogenous phase or using a primer attached to a solid phase, to the extended sequence of which the labeled probe can hybridize and thus, the concentration and/or conformation of which can be determined either specifically at the solid phase support or within the free solution.

a 10. The process according to at least ~~one of claims 1 to 9~~, characterized in that at least one molecule of fluorescent dye is linked to a nucleic acid molecule, the sequence of which is identical or homologous to the nucleic acid to be detected or to the co-amplified nucleic acid standard.

a 11. The process according to at least ~~one of claims 1 to 10~~, characterized in that the nucleic acid molecule linked to the fluorescent dye is added to the reaction mixture after completed amplification, hybridization with the amplified nucleic acids being effected by a thermal denaturation step with a subsequent renaturation step.

a 12. The process according to at least ~~one of claims 1 to 4 and 10~~, characterized in that the nucleic acid molecule linked to the fluorescent dye is added to the reaction mixture prior to completed amplification, the probe being added as a non-amplifiable double-stranded

RNA or as a non-amplifiable chemically modified nucleic acid.

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13. The process according to at least one of claims 1 to 12, characterized in that a primer of a primer pair is used for the amplification, which primer encodes a G:C-rich region at the 5' terminus of preferably from 15 to 20 G:C residues.

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14. The process according to at least one of claims 1 to 10, characterized in that the probe used for determination is an oligo- or polynucleotide having at least two chemical structural elements capable of absorbing or, due to excitation, emitting electromagnetic waves, one of said structural elements being capable of linking or cleaving a stable bond to another position of the oligo- or polynucleotide through the action of electromagnetic waves.

15. The process according to claim 14, wherein the structural element absorbing and/or emitting an electromagnetic wave has a chromophoric system.

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16. The process according to claim 15, wherein the chromophoric system luminesces via dye substituent.

17. The process according to at least one of claims 14 to 16, wherein the structural element linking or cleaving stable bonds under electromagnetic influence is a photochemical crosslinker.

18. The process according to claim 17, wherein the photochemical crosslinker is a psoralene derivative.

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19. The process according to at least one of claims 14 to 18, wherein the spacing of the structural elements

claim 1

claim 1

claim 14

may not be below 8 to 12, preferably 10 nucleotide positions.

- a 20. The process according to at least ~~one of claims 1 to 19~~, characterized in that sheet systems having hollows or recesses as reaction chambers are used which are thermally weldable and suited to accommodate ready-for-use reagent mixtures in lyophilized or matrix-bound form and are suitable for direct optical measurement.
- a 21. The process according to at least ~~one of claims 1 to 20~~, characterized in that the charged reagents are stored in spatially separated matrices, and subsequent to sealing the reaction chamber, are introduced into the reaction process.
- a 22. The process according to at least ~~one of claims 1 to 21~~, characterized in that the distance of the reaction chambers is selected such that evaluation is effected using commercially equipment such as in the form of microtitration equipment.
- a 23. The process according to at least ~~one of claims 1 to 22~~, characterized in that for analysis, the mixture of nucleic acids is subjected to a time-controlled temperature gradient, preferably with linear progression, with the variation of the spectroscopic parameter being monitored as a function of time and/or temperature.)
- a 24. The process according to claim 23, wherein the conformational analysis of the nucleic acid mixture is effected using temperature gel electrophoresis, a chromatographic process, or directly in homogenous solution, or a combination of these processes.
- a 25. The process according to claim 23 and/or 24, characterized in that from the dependence of the optical

parameter with temperature and/or time, the presence and/or number and/or homology of a nucleic acid in question to the corresponding standard may be concluded.

a 26. The process according to at least one of claims 1 to 25, characterized in that data evaluation is effected on-line using a data processing system.

a 27. An oligo- or polynucleotide probe for operating the process according to at least one of claims 1 to 26 and having at least one chemical structural element capable of interacting with electromagnetic waves and/or by absorption or emission of radiation, with cleavage or linkage of stable bonds, wherein said structural element does not represent a purine or pyrimidine substituent of naturally occurring nucleotide components.

28. The probe according to claim 27, wherein the structural element capable of linking stable bonds is psoralene or a psoralene derivative.

a 29. The probe according to one of claims 27 and/or 28, wherein at least one of the structural elements capable of interacting with electromagnetic radiation luminesces.

a 30. The probe according to at least one of claims 26 to 29, wherein the spacing of the oligo- or polynucleotide structural elements is at least from 8 to 12 nucleotides.

a 31. ^{claim 1} A device for operating the process according to one of claims 1 to 30, permitting computer-controlled, time-dependent thermostatting of the reaction compartments.

Claim 1

Claim 26

32. The device according to claim 31, comprising an optical unit, wherein preferably, a laser is employed for excitation and an optical detecting unit for registering the emitted fluorescence signal.

33. A means for operating the process according to at least one of claims 1 to 26, ^{comprising} consisting of a system of reaction compartments, preferably, a sheet system with ready-for-use reagents in lyophilized form, the reaction compartments preferably being arranged with microtitration dimensions.

34. The means for operating the process according to claim 33, characterized in that the reagents are fixated and/or stored in at least one water-soluble matrix.

35. ^{claim 33} The means for operating the process according to claims 33 and/or 34, wherein the matrix contains stabilizers, preferably sugars, particularly trehalose or saccharose.

36. ^{claim 33} The means for operating the process according to at least one of claims 33 to 35, wherein the reaction compartment and/or further reagent reservoirs contain amplification primers, buffer components, at least one polymerase, and co-factors.

37. ^{claim 33} The means for operating the process according to at least one of claims 33 to 36, wherein the sheet sealing the reaction compartment contains at least one further reagent reservoir in a matrix, with the probe preferably being stored therein as well as the reagents required for hybridization.

38. The means according to at least one of claims 33 to 37, composed in kit systems.

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